Seed germination and seedling growth of lettuce after seed pelleting with zinc

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ABSTRACT: Lettuce seeds are flat in shape and small in size with small amounts of stored food reserves resulting in non-uniform germination, and slow seedling growth rate. Therefore, pelleting the seeds for improving germination uniformity, and making it easier to plant was conducted. This research was to study the quality of lettuce seeds after pelleting the seeds with plant nutrients. The experiment was conducted at Seed Quality Testing Laboratory, Seed Processing Plant, Faculty of Agriculture, Khon Kaen University. Then, the germination of lettuce seeds was examined. The results showed that the pelleting seed with $ZnSO_4.7H_2O$ in all rates had not affect on the germination of lettuce seeds. But the pelleted seeds with $ZnSO_4.7H_2O$ in all rates had affected the promotion of the growth of lettuce seedlings. It also found that the length of the shoot and root of the pelleting seeds with $ZnSO_4.7H_2O$ at the rate of 0.6 grams was longer than the non-pelleted seeds. In accelerated ageing of seeds, every process had a pelleting method with a percentage of germination better than the seeds without pelleting both under the laboratory and greenhouse conditions.
Keywords: seed enhancement, seed germination, zinc sulfate, micronutrients

Introduction

Lettuce (*Lactuca sativa* L.) is a vegetable leaf-eating species that are important to the economy. By the year 2558, Thailand exported lettuce seeds of 10.35 tons, worth 7.68 billion Baht (Thai seed trade association, 2015). Many types of lettuce production, the nursery is a very important procedure in the production system. As the lettuce seeds are small and have small amounts of stored food reserves, and low germination rate, therefore it is important to have nursery seedlings to germinate them regularly (Kangsopa and Siri, 2015). Also, pelleting the seeds which currently raising the level of quality of seeds and very widely used. Pelleting the seeds is the method that makes the seeds are covered, and change the shapes from the original. Moreover, the pelleting seeds can increase more plant nutrients which are useful to the germination and the growth development of seedlings to stick with the seed (Siri, 2015). One of the plant nutrients that critical for the seedlings is the zinc, which greatly needed for various biochemical processes of the plant in the duration of seedlings, including photosynthesis, sugar production, protein synthesis, reproduction, growing steadily, and disease resistance (Wiatrak, 2013). However, the pelleting seeds will be successful if plant nutrients are chosen properly in term of the type and the rate of plant nutrients. For example, the report of Adhikari et al. (2016) found that coated seed with Zn at the rate of 50 mg showed the corn, soy beans, pigeon and ladies finger had dry weight of plants better than uncoated seed, including 22.35, 10.73, 14.60 and 9.47 grams, respectively. In addition, in each type of plant, there are different accumulated amount of zinc (Zn). Indeed that seed improvement with plant nutrients can enhance the quality of seeds.

Therefore, the purpose of this experiment was to study the changes of germination, vigor, and growth after pelleting the lettuce seeds with elemental zinc.

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Materials and methods

Seed material

Hybrid lettuce seeds (RUTLL58-1) (*Lactuca sativa*) were obtained from the Faculty of Sciences and Agricultural Technology, Rajamangala University of Technology Lanna Lampang, Lampang, Thailand.

Seed pelleting process

The pelleting seeds, 50 grams of calcium carbonate (CC) were used for forming a pellet (Layer 1), then adding 200 grams of calcium sulfate (CS) (Layer 2) for pelleting materials. Using 0.3% carboxymethyl cellulose (CMC; Sigma Aldrich) as a binder, and Zinc Sulfate 7-Hydrate Crystal ($ZnSO_4.7H_2O$; JT. Baker®) was used as an active ingredient. The method of the pelleting seeds with plant nutrients consisted of 5 methods: Control (non-pelleted seed) T1), pelleted seed with CC+CS T2), and pelleted seeds with CC+CS mixed with $ZnSO_4.7H_2O$ at the rate of 0.2, 0.4 and 0.6 grams (T3, T4 and T5 respectively). The pelleting process with a Rotary Drum (Model SKK12, Seed Processing Plant, Khon Kaen University), then take the seeds that passed through pelleting to reduce the moisture with Air Dryer Dehumidifier SKK09 to have moisture equivalent to the moisture content of seeds before pelleting process (5-6%).

Seed measurement

Laboratory conditions germination test: the germination percentage of seeds was tested by conducting a simple random sampling seed passed through pelleting process and non-pelleting. Each trial process, 50 pellets were taken in each time to test germination for 4 replicates by using the top of paper germination method. Then, the seeds were taken to Seed Germination Incubator, and counting germination after planting at 4-7 days to evaluate the results of germination examination according to the ISTA (2013).

Greenhouse conditions germination test: the examination of greenhouse condition was conducted by using peat moss, which is supplemented material, 4 replicates with 50 pellets in each time, then examined and counted germination, and evaluated using the same tests under laboratory condition.

Speed of germination test: speed of germination was tested under the ISTA rules (2013) and then counted the number of seeds that germinate into normal seedlings, and the number of days germinating, as well as the number of days germinating from the first counted until the final counted. However, the testing under laboratory condition, and the testing under greenhouse condition were evaluated as well.

Shoot and root length test: the examination of the shoot and root length was carried out by randomly examined the seedlings at 14 days after planting for 4 replicates, 10 seedlings were examined in each time. Then, they were taken to measure the shoot length and root length (mm).

Fresh weight and dry weight under laboratory and greenhouse conditions test: the examination of fresh weight and dry weight of seedlings was conducted by seedlings at 14 days after planting for 4 replicates, 10 seedlings were determined following oven drying at 60 °C for 72 hours and then be weighed and recorded.

Accelerate ageing test: All seed treatments were exposed to accelerate ageing in a humidity chamber, 40 °C and 100% relative humidity (RH), for 48 h. Germination testing was conducted under the laboratory and greenhouse conditions.

Experimental design

Percentage of germination was arcsine-transformed to normalize data before statistical

analyses. All data were analyzed by one-way ANOVA (complete randomized design) and the difference between treatments was tested by Duncan's new multiple range test (DMRT).

Results and Discussion

Quality of lettuce seeds after pelleting the seeds with zinc

After tested under laboratory condition, it was found that the germination and speed of germination were not significantly different. The comparison between the lettuce seeds (control) and the group of pelleting seeds demonstrated that the pelleted seeds alone and pelleted seeds with plant nutrients has not affected on the germination and the vigor of lettuce seeds. The examination under greenhouse condition showed that the germination and speed of germination after the tested were not significantly different. The results were observed clearly from the tested that the trend of lettuce seeds quality after pelleting seeds with $ZnSO_4.7H_2O$ at all rates was likely better than non-pelleted seeds **(Table 1)**. Generally, zinc is essential for all plants. Zinc is a substance that helps the enzyme (Taiz and Zeiger, 1994) in the biochemical reaction of the plant, including photosynthesis, creating the sugar, protein synthesis, seedling growth, transport of the plant hormone auxin and disease resistance (Bhalerao et al., 2002; Kaya and Higgs, 2002; Smet et al., 2007). Therefore, the direct response on the promotion of germination of lettuce seeds may not be clearly seen in the results. The report of Korishettar et al. (2016) showed that coating pigeon pea seeds with nanoparticles of iron at a rate of 750 ppm had not affected results on seed quality and also helped a pigeon pea to have germination at the highest (96%).

The growth of lettuce seeds after pelleting the seeds with plant nutrients

When examined the growth of lettuce seedlings under laboratory condition, the results showed that the pelleted seeds with 0.6 grams of $ZnSO_4.7H_2O$ had the best shoot length and root length 92.0 and 129.66 mm, respectively **(Figure 1a,b)**. There were statistically significant differences when comparing non pelleted seeds (control) with other methods. When examined a fresh weight and dry weight of seedlings, it was also found that the pelleted seeds with $ZnSO_4.7H_2O$ rate of 0.6 grams had the fresh weight and dry of seedlings better than the non-pelleted seeds and pelleted seeds other methods **(Figure 1c,d; Figure 2a,b)**.

Table 1 Germination percentage and speed of germination of lettuce pelleted seed after tested under laboratory and greenhouse conditions.

Treatments	Laboratory condition		Greenhouse condition	
	Germination (%)	Speed of germination (Seedling/day)	Germination (%)	Speed of germination (Seedling/day)
Control	98[1/]	24.67	97	24.40
Pelleted seed (P)	99	24.81	98	24.77
(P) + $ZnSO_4$ (0.2 g)	98	24.63	98	24.75
(P) + $ZnSO_4$ (0.4 g)	98	24.69	99	24.84
(P) + $ZnSO_4$ (0.6 g)	99	24.71	100	24.78
F-test	NS	NS	NS	NS
C.V. (%)	0.97	0.89	0.93	1.01

NS: not significantly different.

[1/]Data are transforming by the arsine before statistical analysis.

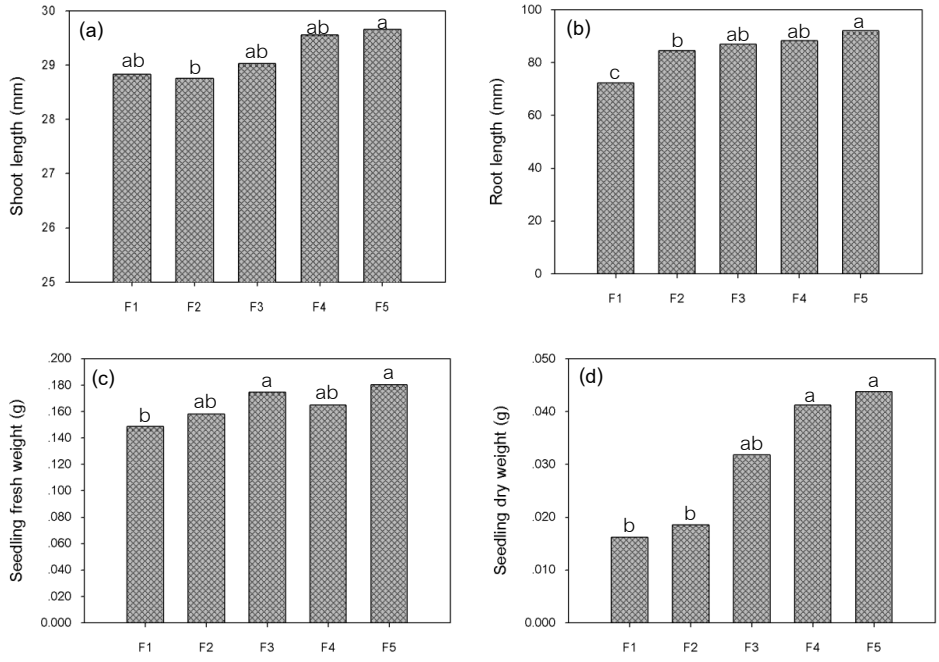

Figure 1 Effect of changes in shoot length (a); root length (b); seedling fresh weight (c) and seedling dry weight (d) after pelleted seeds with zinc difference rate under laboratory conditions. F1: Control, F2: Pelleted seed, F3: Pelleted seed + $ZnSO_4$ (0.2 g), F4: Pelleted seed + $ZnSO_4$ (0.4 g), F5: Pelleted seed + $ZnSO_4$ (0.6 g).

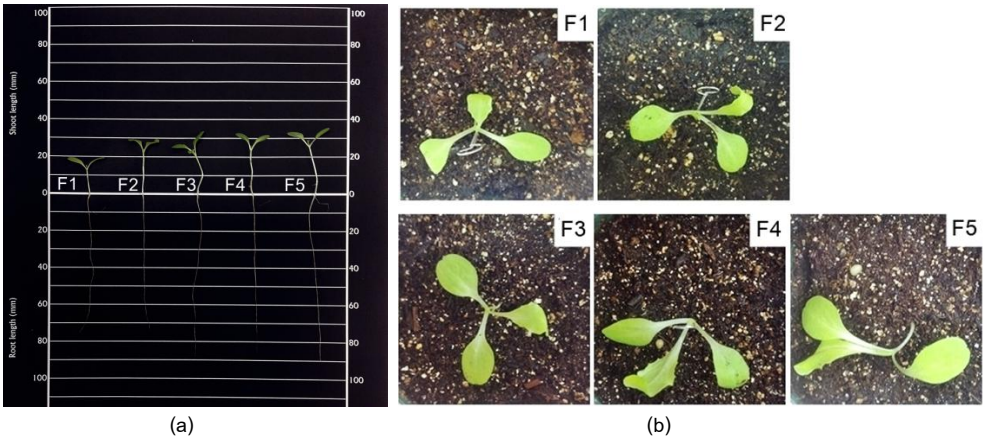

Figure 2 The growth of lettuce seedling 14 days after planting. Effect of seed pelleting mixed $ZnSO_4.7H_2O$ after tested under laboratory condition (a) and greenhouse condition (b): Control (non-pelleted seeds) T1); pelleted seed T2); seed pelleting mixed $ZnSO_4.7H_2O$ rate 0.2 g., 0.4 g., 0.6 g. (T3, T4, T5) respectively.

The growth of lettuce seedlings under greenhouse conditions demonstrated that the pelleted seeds with 0.6 grams of zinc sulfate still had the shoot length, fresh weight and dry weight were better and significant differences when comparing with control and pelleted seeds other methods **(Figure 3a,b,c)**. The experimental results were apparently seen that the pelleted seed with

zinc sulfate all rates had effects upon promoting the growth of seedlings rather than seeds of control, especially, the pelleted seeds with 0.6 grams of $ZnSO_4.7H_2O$. In the meanwhile, zinc to be is essential for the development of growth and helps promote the seedlings to be resistant to various diseases as well (Kaya and Higgs, 2002). In order for to help the enzyme system to stimulate growth, as well as helping to strengthen the creation of chlorophyll and essential part of moving carbohydrates and stimulate the use of sugar in plants, Zinc has a direct effect on favorable terms to promote the growth of lettuce seedlings, both extending root cells and shoot of seedlings (Zhang and Forde, 1998). Therefore, the advantages of the seed pelleting technology with plant nutrients can promote lettuce seedlings after germination bring dissolved zinc adhering to the pellet around the roots of seedlings to use in order to develop as vigor and healthy seedlings immediately. The important reason of this research for using $ZnSO_4.7H_2O$ was that the plants absorb zinc in the form of Zn^{+2} and the chemicals that the plants want to use often in compounds of $ZnSO_4$. Therefore, the selection of using $ZnSO_4.7H_2O$ increases the efficiency and convenience to plant seedlings. It makes the plant absorb zinc in the form of Zn^{+2} and assist in the growth immediately. It is clearly showed that the technology in pelleting the seeds with zinc can help to increase the growth of lettuce seedlings of better than lettuce seeds that were not pelleted.

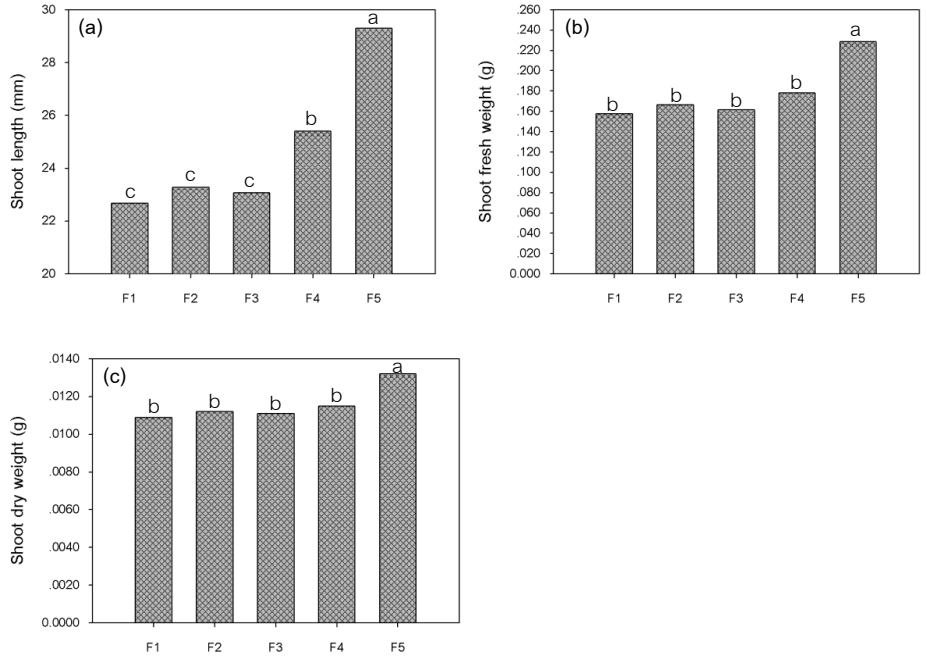

Figure 3 Effect of changes in shoot length (a); shoot fresh weight (b) and shoot dry weight (c) after pelleted seeds with zinc difference rate under greenhouse conditions. F1: Control, F2: Pelleted seed, F3: Pelleted seed + $ZnSO_4$ (0.2 g), F4: Pelleted seed + $ZnSO_4$ (0.4 g), F5: Pelleted seed + $ZnSO_4$ (0.6 g).

In addition, the results many research studies also showed the advantages of using zinc to improve other types of seed for example, the report of Masuthi et al. (2009) found that the pelleting seeds of cowpea (*Vigna unguiculata* L.) with $ZnSO_4$ at a rate of 250 mg/kg seed. The results clearly that pelleting the seeds with zinc (Zn) has increased the growth and productivity higher than non-pelleted seeds. Additionally, in sunflower seed Suman (2002) reported that the seed treatment with $ZnSO_4$ yielded increasingly from the original, and the germination increased to 5.66 percent, root length (20.91 cm), shoot length (17.11 cm) and vigor index (36.37). Furthermore, the report of Shabaz et al. (2015) revealed that the coated corn seed with $ZnSO_4$ at a rate of 2 g/kg of seeds has resulted the shoot and root length of maize seedlings better than uncoated seed. Adhikari et al. (2016) reported that the seed of the corn, soybeans, pigeon beans and ladies finger that coated with 50 mg of zinc had dry weight, the plants were better than uncoated seed; 22.35 g, 10.73 g, 14.60 g. and 9.47 g., respectively. In addition, it was found that each type of plants had an accumulated amount of zinc better than other experimental methods, and also found that zinc could increase rice yields from the original one (Maharana et al., 1993).

Lettuce seed quality after accelerated ageing

Accelerate ageing of seeds is the technique using to test the vigor of the seed, the germination of seeds is reduced slowly or quickly based on the deterioration of the seeds because accelerated ageing of seeds are placed under high temperature conditions. The seeds are accelerated breathing process and the seeds will release the heat and moisture (Delouche and Baskin, 1973). Therefore, the seeds have deteriorated more quickly. The results of the experiment obviously showed that the method of the pelleted seeds had the percentage of germination better than the seeds that were not pelleted both under the laboratory and greenhouse conditions. In addition, it was statistically different under the laboratory, the pelleted seeds with $ZnSO_4$ at the rates of 0.4 and 0.6 grams, there was a higher germination rates than other methods as in the greenhouse **(Table 2)**. The pelleted seeds with $ZnSO_4$ at the rates of 0.4 and 0.6 grams were likely to remain higher germination than other methods but it was not statistically different compared with the same pelleting methods.

Table 2 Germination percentages and speed of germination of lettuce seeds tested under laboratory and greenhouse conditions after pelleting process and accelerated ageing.

Treatments	Laboratory condition		Greenhouse condition	
	Germination (%)	Speed of germination (Seedling/day)	Germination (%)	Speed of germination (Seedling/day)
Control	73 b[1/2/]	20.50 a	67 b	15.28 a
Pelleted seed (P)	81 ab	18.71 ab	79 a	13.17 ab
(P) + $ZnSO_4$ (0.2 g)	76 b	17.33 b	77 a	12.75 b
(P) + $ZnSO_4$ (0.4 g)	84 a	18.37 ab	83 a	13.94 ab
(P) + $ZnSO_4$ (0.6 g)	86 a	19.60 ab	82 a	13.28 ab
F-test	*	*	*	*
C.V. (%)	6.48	9.89	8.19	10.62

*: Significantly different at P≤0.05.

[1/]Means within a column followed by the same letter are not significantly at P<0.01 by DMRT

[2/]Data are transforming by the arsine before statistical analysis.

The experimental results were apparently seen that of the seed pelleting method could protect the seed from the environment where inappropriate like wearing the armor to protect lettuce seeds. It helped slow down the heat and moisture over a period of time. When examined the speed of germination, it was found that both under the laboratory and greenhouse conditions, the non-pelleted seed had the best speed of germination at 20.50 and 15.28 seedling/days respectively and there was statistically different. When comparing with the pelleting groups, it was found that the pelleted seeds with $ZnSO_4$ at the rate of 0.4 and 0.6 g. tend to have the speed of germination better than other seed pelleting methods.

The trial results of lettuce seeds can germinate more quickly than the pelleted seeds. Although the non-pelleted seeds can germinate more quickly than the pelleted seeds, the non-pelleted seeds have germination percentage decreased. The pelleted seeds of all pelleting methods had slow speed of germination but it had not affected the germination percentage of lettuce seeds. In addition, the seeds germinated normal seedlings could bring nutrients with the pelleted substance to use for growing. Therefore, pelleted seeds with plant nutrients made the seedlings after accelerated ageing of seeds grow better than the non-pelleted seeds. The results were consistent with the report of Buakaew and Siri (2016) in which after accelerated ageing of lettuce seeds, the seeds had better growth than non-pelleted seeds both the examination under the laboratory and greenhouse conditions. There are reports related pelleting tobacco seeds showed that pelleted tobacco seeds had better germination percentage than non-pelleted seeds. It was also found that the tobacco seeds were pelleted with $MgSO_4$ and KCl has affected the germination percentage of tobacco seeds for every pelleting method greater than non-pelleted seeds both under the laboratory and greenhouse conditions (Trachoo et al., 2016). Other from this after accelerated ageing the pelleted seeds with different fillers and binder materials still not affecting on the germination percentage of tobacco seeds (Kangsopa and Siri, 2014a; Kangsopa and Siri, 2014b).

Conclusions

1. Seed pelleting with $ZnSO_4.7H_2O$ had not affected seed germination and speed of germination on lettuce seeds.
2. Seed pelleting with a rate of 0.6 grams of $ZnSO_4.7H_2O$ had the best growth of lettuce seedlings when testing both under laboratory and greenhouse conditions.
3. After accelerated ageing, it was found that the pelleted seeds with $ZnSO_4.7H_2O$ at all the rates still had the germination percentage better than the non-pelleted seeds both under laboratory and greenhouse conditions.

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